Bio type®
Diagnostic GmbH

Mentype® DIPscreen
Manual

The entry to a quantitative chimerism analysis

In-Vitro-Diagnostics

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Made in Germany

Biotype Diagnostic GmbH develops, produces and markets their PCR-based rapid Mentype® Detection Kits. Our products provide customers with fast and reliable testing methods for professional medical diagnostics.

Our Mentype® Test Kits guarantee highest quality standards for clinical research and diagnostics.

For information and enquiries about the Mentype® **DIPscreen** please do not hesitate to get in touch or visit www.biotype.de/en/home.html.

Product description

Mentype® DIPscreen is a multiplex-PCR application developed to identify DIP polymorphisms that individually occur in donor or recipient, and, constitute informative loci. In a single multiplex-PCR 33 DIP-loci are simultaneously screened together with the gender specific locus Amelogenin. Mentype® DIPscreen is a multiplex-PCR application that mediates monitoring of chimerism samples after stem cell transplatation. The flexible assay format allows an individual diagnostics at any time required.

Analysis of molecular chimerism resulting from allogeneic stem cell transplantation has become a well established method to control the course of transplant engraftment and to assess the risk of threatening relapse. Molecular chimerism analysis can be performed on diverse DNA-sequence motifs of which biallelic short insertion/deletion polymorphisms (DIPs, INDELs) offer substantial benefits. Polymerase-mediated amplification of DIP-markers does not result in formation of stutter peaks that can hamper clear analysis. Moreover, these polymorphisms are best suited for allele specific quantitative approaches. Mentype® DIPscreen is a DIP-based chimerism analysis and therefore accounts for an unambiguous donor/recipient differentiation and highly clear chimerims monitoring.

Identified informative DIP-loci can subsequently be addressed by Mentype® DIPquant specific real-time PCR assays to approach highly quantitative chimerism monitoring.

The 33 DIP loci addressed by Mentype® DIPscreen are distributed over 18 chromosomes, and are at least separated by 10 Mbp each (see Tab. 1). The detection limit of Mentype® DIPscreen is about 200 pg genomic DNA. The optimal range under standard conditions is 1.0 –2.0 ng DNA. For fast and sensitive fragment length analysis primers are fluorescence-labelled with 6–FAM, BTG, or BTY.

The test kit was validated and evaluated using the GeneAmp® 9700 Silver, Eppendorf Mastercycler ep-S, Biometra T1, ABI PRISM® 3130 Genetic Analyzer running with 36 cm capillary array and POP4® polymer. Development, manufacture and distribution of Biotype® products are certified according to DIN EN ISO13485.

Content

1. Description of the Mentype® DIPscreen

Table 1. Locus–specific information **of Mentype® DIPscreen**

DIP Locus	Chromosomal position	Motive (–DIP / +DIP)
FAM Panel		
AM X	Xp22.1-22.3	
AM Y	Yp11.2	
HLD106	16q13	-/AATGCGT
HLD70	6q16.1	-/AGCA
HLD84	8q24.12	-/CTTTC
HLD103	12q23.1	-/GCTTATAA
HLD104	13q32.1	-/ACTC
HLD116	18p11.22	-/AGGTGTCGAACAACATGATAC
HLD112	17p12	-/TTGTA
HLD307	Xp11.23	-/TCAACCAA
HLD310	2p22.3	-/GTCTGGTT
HLD110	16q22.1	-/TCCCTG
HLD133	3p22.1	-/CAACCTGGATT
HLD79	7q31.2	-/AATCT
HLD105	14q24.3	-/ATAGACAA
HLD140	3q23	-/GGTAGTATGGGCCT
HLD163	12q24.31	-/AACTACGGCACGCCC
BTG Panel		
HLD91	11q14.1	-/GATA
HLD23	18p11.32	-/CTTTAA
HLD88	9q22.33	-/CCACAAAGA
HLD101	15q26.1	-/GTAG
HLD67	5q33.3	-/CTACTGAC
HLD301	17q21.32	-/CAGGGGCTC
HLD53	3q22.1	-/ATGT
HLD97	13q13.1	-/AGAGAAAGCTGAAG
HLD152	16p13.2	-/TGGTCAAAGGCA
HLD128	1q31.3	-/ATTAAATA
HLD134	5q11.2	-/ATGATGGTTCTTCAGA
HLD305	20q11.22	-/CAAGGTCCCACCACACTCGCGTGGGA
BTY Panel		
HLD48	2q11.2	-/GACTT
HLD114	17p13.2	-/TCCTATTCTACTCTGAAT
HLD304	9q34.3	-/GAGCTGCTCAAGAGAGAGG
HLD131	7q36.2	-/TTGGGCTTATT
HLD38	1q32.2	-/TAGTT
HLD82	7q21.3	-ACCTCCTACTCCTTGGTCTATTCCTGGTCACATGTACT

Abbreviations: HLD = Human Locus DIP, -DIP = Deletion, +DIP = Insertion

Table 1 shows the chromosomal position, motif and respective reference allele of DIP-loci addressed by Mentype® DIPscreen.

Kit Content

Mentype® DIPscreen (100 Reactions)

Nuclease-free water	3.0 ml
Reaction mix A	500 µl
Primer mix	500 µl
Multi Taq2 DNA polymerase	60 µl
Control DNA XY13 (2ng/µl)	10 µl
DNA Size Standard 550 (BTO)	50 µl
Allelic ladder	25 µl

Ordering information

Mentype® DIPscreen	25 reactions	Cat. No.	45-45410-0025
Mentype® DIPscreen	100 reactions	Cat. No.	45-45410-0100
Mentype® DIPscreen	400 reactions	Cat. No.	45-45410-0400

Storage

Store all components at -20 °C and avoid repeated thawing and freezing. Primer mix and allelic ladder must be stored protected from light. The DNA samples and post-PCR reagents (allelic ladder and DNA size standard) should be stored separately from the PCR reagents. The expiry date is indicated on the kit cover.

Additionally required reagents

Additional reagents are required in order to use the Biotype® PCR Amplification Kit:

Reagent	Supplier	Order Number
Hi-Di™ Formamide, 25 ml	Applied Biosystems	4311320
Matrix Standards BT5 single-capillary instruments (5x25 µl)	Biotype Diagnostic GmbH	00-10411-0025
Matrix Standards BT5 multi-capillary instruments (25 µl)	Biotype Diagnostic GmbH	00-10421-0025
Matrix Standards BT5 multi-capillary instruments (50 µl)	Biotype Diagnostic GmbH	00-10421-0050

Warning and safety instructions

The PCR Amplification Kit contains the following potentially hazardous chemicals:

Kit component	Chemical	Hazards
Reaction mix	Sodium azide NaN_3	toxic if swallowed, develops toxic gases when it gets in contact with acids

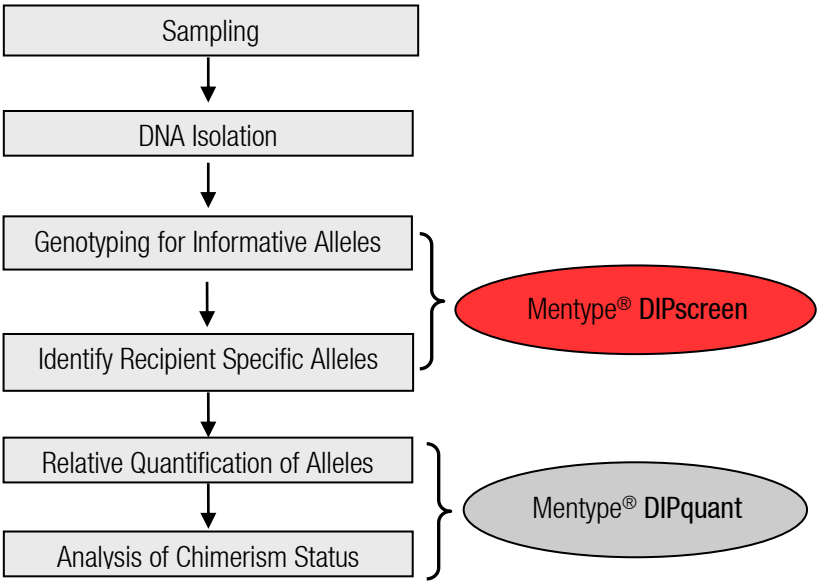
Observe the Material Safety Data Sheets (MSDS) for all Biotype® products, which are available on request. Please contact the respective manufacturers for copies of the MSDS for any additionally needed reagents.

Quality assurance

All kit components undergo an intensive quality assurance process at Biotype Diagnostic GmbH. The quality of the test kits is permanently monitored to ensure unrestricted usability. Please contact us if you have any questions regarding quality assurance.

Trademarks and patents

Outline of working steps performed with Mentype® DIP–products



From sample to analysis – Monitoring chimerism with the Mentype® **DIPscreen** and the Mentype® **DIPquant** assay

Protocols for PCR amplification, electrophoresis and analysis

2. PCR amplification

2.1 Master mix preparation

The table below shows the volumes of all PCR reagents per 25 µl reaction volume, including a sample volume of 1.0 µl (template DNA). The number of reactions to be set up shall be determined taking into account positive and negative control reactions. Add one or two reactions to this number to compensate the pipetting error.

Component	Volume
Nuclease-free water	13.4 µl
Reaction mix A*	5.0 µl
Primer mix	5.0 µl
Multi Taq2 DNA polymerase (hot start, 2.5 U/µl)	0.6 µl
Volume of master mix	24.0 µl

* contains Mg^{2+}, dNTPs, BSA

All components should be mixed (vortex) and centrifuged for about 10 s before preparing the master mix. The DNA volume applied to the assay depends on its concentration. For reference samples 1 µl is mostly sufficient. For critical patient samples the amount of template can be increased appropriately. Fill up the final reaction volume to 25 µl with nuclease-free water.

Generally, DNA templates shall be stored in nuclease-free water or in diluted TE buffer (10 mM Tris HCl, pH 8.0 and 1 mM EDTA), e.g. 0.1x TE buffer.

The primer mixes are adjusted for balanced peak heights at 28 PCR cycles and 1 ng Control DNA XY13 in a reaction volume of 25 µl. If more DNA template is applied, higher peaks can be expected for small PCR fragments and relatively low peaks for large fragments. Reduce the amount of DNA template to correct this imbalance.

Positive control

For the positive amplification control, dilute the Control DNA XY13 to 1 ng/µl. Instead of the template DNA, pipette the diluted Control DNA into a reaction tube containing the PCR master mix.

Negative control

For the negative amplification control, pipette nuclease-free water instead of template DNA into a reaction tube that contains the PCR master mix.

Template DNA

Sometimes, measured DNA concentration varies depending on the quantification method used. It might thus be necessary to adjust the optimal DNA amount.

2.2 PCR amplification parameter

Perform a “hot start” PCR in order to activate the Multi Taq2 DNA Polymerase and to prevent formation of non-specific amplification products.

The number of cycles depends on the amount of DNA applied. 28 PCR cycles are recommended for all samples.

Standard method

Recommended for all DNA samples

Temperature	Time	
94°C	4 min (hot start for activation of the Multi Taq2 DNA polymerase)	
94°C	30 s	
60°C	**120 s**	**28 cycles**
72°C	75 s	
68°C	60 min	
10°C	∞	hold

Note: To provide an optimal kit balance the ramping rate of the thermal cycler should be adjusted to 4-5 °C/s.

Very small amounts of DNA may result in statistical dropouts and imbalances of the peaks. Increasing numbers of PCR cycles raise the risk of cross contamination caused by minimal amounts of impurities. Furthermore, unspecific amplification products could appear.

3. Electrophoresis using the ABI PRISM® 310 Genetic Analyzer

For general instructions on instrument setup, matrix generation and application of the GeneScan® or GeneMapper® ID software, refer to the *ABI PRISM® 310 Genetic Analyzer User's Manual*. Electrophoresis using the GeneScan® software is described below.

The virtual **filter set G5** shall be used for combined application of the five fluorescent labels **6–FAM**, **BTG**, **BTY, BTR, and BTO** (the matrix standard will be called **BT5** hereinafter).

Material	
Capillary	47 cm / 50 µm (green)
Polymer	POP4® for 310 Genetic Analyzer
Buffer	10x Genetic Analyzer Buffer with EDTA

3.1 Matrix generation

Prior to conducting DNA fragment size analysis with the **Filter Set G5**, a matrix with the five fluorescent labels **6–FAM**, **BTG**, **BTY**, **BTR**, and **BTO** must be generated.

Color	Matrix standard
Blue (B)	6-FAM
Green (G)	BTG
Yellow (Y)	BTY
Red (R)	BTR
Orange (O)	BTO

Five electrophoresis runs shall be conducted, one for each fluorescent label, **6–FAM**, **BTG**, **BTY**, **BTR**, and **BTO**. Use the same conditions as for samples and allelic ladders of the Biotype® test kit to generate suitable matrix files.

Matrix sample	Component	Volume
Matrix sample 1	Hi-Di™ Formamide	12.0 µl
	Matrix standard **6–FAM**	1.0 µl
Matrix sample 2	Hi-Di™ Formamide	12.0 µl
	Matrix standard **BTG**	1.0 µl
Matrix sample 3	Hi-Di™ Formamide	12.0 µl
	Matrix standard **BTY**	1.0 µl
Matrix sample 4	Hi-Di™ Formamide	12.0 µl
	Matrix standard **BTR**	1.0 µl
Matrix sample 5	Hi-Di™ Formamide	12.0 µl
	Matrix standard **BTO**	1.0 µl

- Denaturation for 3 min at 95°C
- Cool down to 4°C and place samples on the autosampler tray

- Create a **Sample Sheet** choose **5 Dyes** and enter a sample designation

Injection list for matrix generation

Parameter	Set up
Module File	GS STR POP4 (1 ml) **G5**
Matrix File	**NONE**
Size Standard*	**NONE**
Injection [s]	5
Injection [kV]	15.0
Run [kV]	15.0
Run [°C]	60
Run Time [min]	24

* Prepare matrix standards always **without DNA Size Standard (BTO)**

Analysis of the matrix samples

- Run the GeneScan® software
- **File** → **New** → **Project** (open folder of current run) → **Add Sample Files**
- Select a matrix sample in the **Sample File** column
- **Sample** → **Raw Data**
- Check the matrix samples for a flat baseline. As shown in the figure below there should be at least five peaks with peak heights about 1000-4000 RFU (Y-axis) for each matrix sample (optimal range: 2000-4000 RFU)

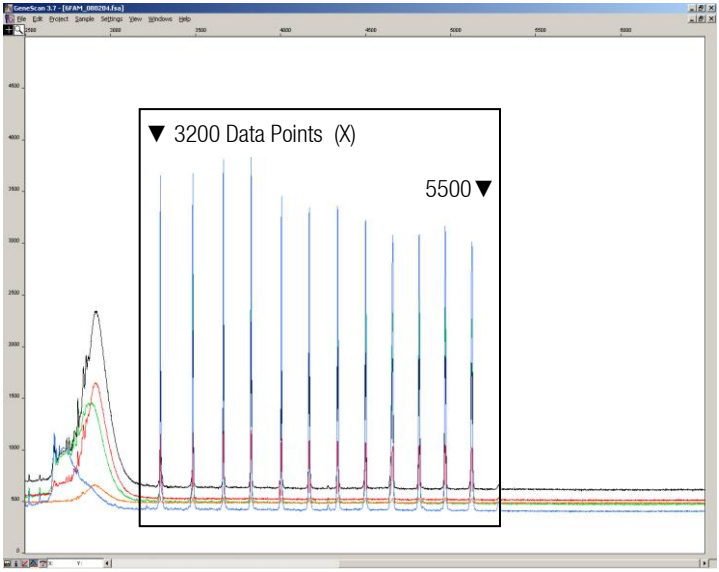


Fig. 1 Electropherogram with raw data of the matrix standard 6-FAM

- Select an analysis range with flat baseline and re-inject the matrix sample if necessary
- Note down start and end value (data points) of the analysis range, e.g. start value 3200, end value 5500
- Calculate the difference, e.g. 5500-3200 = 2300 data points

Generation of a new matrix

- File → New → Matrix

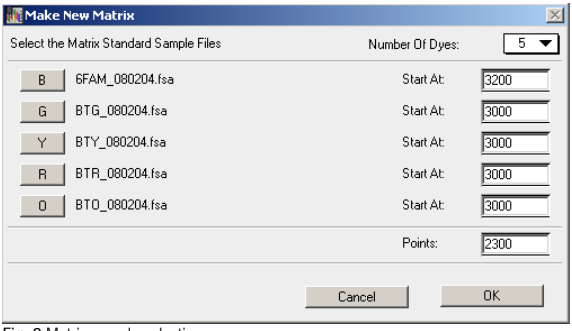


Fig. 2 Matrix sample selection

- Import matrix samples for all dyes (B, G, Y, R, O)
- Enter a **Start At** value, e.g. 3200
- Enter the calculated difference under **Points**, e.g. 2300
- Click on **OK** to calculate the new matrix

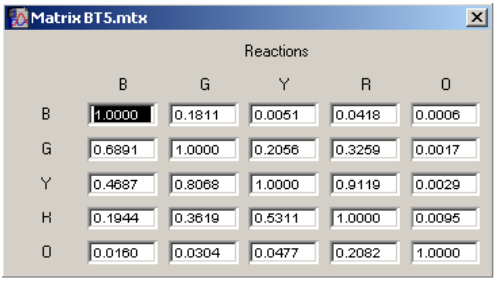


Fig. 3 New matrix BT5

- Save the matrix in the matrix folder: **File → Save as**, e.g. Matrix BT5

Matrix check

Check the new matrix with current samples.
- **File → New → Project** (open folder of the respective run) → **Add Sample Files**
- Select sample(s) in the **Sample File** column
- **Sample → Install New Matrix** (open matrix folder and select new matrix)
- Re-analyse your samples

There should be no pull-up peaks between the dye panels (B, G, Y, R, O) with the new matrix.

3.2 Sample preparation

Component	Volume
Hi-Di™ Formamide	12.0 µl
DNA Size Standard 550 (BTO)	0.5 µl
Prepare 12 µl of the mix (formamide + DNA size standard) for all samples	
Add 1 µl PCR product (diluted if necessary) or allelic ladder	

- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place samples on the autosampler tray

Signal intensities

Options to increase the signal intensity:

- Reduce the volume of the DNA Size Standard 550 (BTO) to peak heights of about 500 relative fluorescent units (RFU)
- Purify the PCR products before starting the analysis

3.3 Setting up the Data Collection Software

- Create a **Sample Sheet** and enter a sample designation

Injection list

Parameter	Set up
Module File	GS STR POP4 (1 ml) **G5**
Matrix File	e.g. Matrix BT5
Size Standard	e.g. SST-BTO_60-450bp
Injection [s]*	5
Injection [kV]	15.0
Run [kV]	15.0
Run [°C]	60
Run Time [min]**	**26**

* Deviating from the standard settings, the injection time may range between 1 and 20 s depending on the type of sample. If reference samples with very high signal intensities are recorded, a shorter injection time may be selected in order to avoid pull-up peaks. For samples with low DNA content an injection time of up to 20 s may be necessary.

** Depending on the analysis conditions, the run time for Mentype® **DIPscreen** was modified in order to be able to analyse fragments with lengths of up to **450 bp**.

3.4 Analysis parameter

The recommended analysis parameters are:

Analysis Range	Full Range
Data Processing	Baseline: Checked Multicomponent: Checked Smooth Options: Light
Peak Detection	Peak Amplitude Thresholds B:* Y:* G:* R:* O:* Min. Peak Half Width: 2 pts Polynominal Degree: 3 Peak Window Size: 15 pts
Size Call Range	Min: 60 Max: 550
Size Calling Method	Local Southern Method
Split Peak Correction	None

* The peak amplitude threshold (cutoff value) corresponds to the minimum peak height that will be detected by the GeneScan® or GeneMapper® ID software. Thresholds are usually 50-200 RFU and should be determined individually by the laboratory. Recommendation: The minimal peak height should be three times as high as the background noise of the baseline.

4. Electrophoresis using the ABI PRISM® 3100–Avant/3100 Genetic Analyzer

For detailed instructions on instrument setup, spectral calibration, application of the ABI PRISM® 3100 Data Collection Software version 1.01 or 1.1 and the GeneScan® software, refer to the *ABI PRISM® 3100-Avant/3100 Genetic Analyzer User's Manual*. For systems with Data Collection Software 2.0 or 3.0 refer to chapter 5.

The system with 4 capillaries is named ABI 3100-Avant, and the system with 16 capillaries is named ABI 3100.

The virtual **filter set G5** shall be used for combined application of the five fluorescent labels **6–FAM**, **BTG**, **BTY**, **BTR**, **and BT0** (the matrix standard will be called **BT5** hereinafter).

Material	
Capillary	36 cm Capillary Array for 3100-Avant/3100
Polymer	POP-4® Polymer for 3100
Buffer	10x Genetic Analyzer Buffer with EDTA

4.1 Spectral calibration / matrix generation

Proper spectral calibration is critical to evaluate multicolour systems with the ABI PRISM® 3100-Avant/3100 Genetic Analyzer and shall be done prior to conducting fragment length analysis with the five fluorescent labels **6–FAM**, **BTG**, **BTY**, **BTR**, and **BT0**. The calibration procedure creates a matrix which is used to correct the overlapping of fluorescence emission spectra of the dyes.

Spectral calibration comprises the following steps:
- Preparation of the spectral calibration standards
- Loading the standards to the 96-well reaction plate (one sample per capillary)
- Entering the plate composition
- Performing a spectral calibration run and checking the matrix

Setting up the spectral calibration standards

Example for 4 capillaries / ABI 3100-Avant

Component	**Volume**
Hi-Di™ Formamide	60.0 µl
Matrix standard **BT5**	5.0 µl

- Load 12 µl of the mix to a 96-well reaction plate, e.g. position **A1–D1**
- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place samples on the autosampler tray

Example for 16 capillaries / ABI 3100

Component	**Volume**
Hi-Di™ Formamide	204.0 µl
Matrix standard **BT5**	17.0 µl

- Load 12 µl of the mix to a 96-well reaction plate, e.g. position **A1–H1** and **A2–H2**
- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place samples on the autosampler tray

Performing a spectral calibration run

First of all, the parameter file for **DyeSetG5** must be modified once to achieve successful calibration with the Data Collection software version 1.0.1 or 1.1.

Spectral parameter

To change settings in the parameter file go to the following path:
D:\AppliedBio\Support Files\Data Collection Support Files\CalibrationData\Spectral Calibration\ParamFiles

- Select **MtxStd{Genescan_SetG5}** to open the PAR-file
- Change **Condition Bounds Range** to [1.0; 20.0]
- Select **File** → **Save As** to save the parameter file under a new name, e.g. MtxStd{Genescan_SetG5_BT5}.par

Always use this parameter file for spectral calibration runs using Biotype® matrix standards **BT5**.

Plate Editor for spectral calibration (I)

- Place the 96-well plate on the autosampler tray
- Run the ABI PRISM® 3100 Data Collection software
- In **Plate View** click **New** to open the **Plate Editor** dialog box
- Enter a name of the plate
- Select **Spectral Calibration**
- Select **96–Well** as plate type and click on **Finish**

Plate editor for spectral calibration (II)

Parameter	Set up
Sample Name	Type name for the matrix samples
Dye Set	G5
Spectral Run Module	*Default* (e.g. Spect36_POP4®)
Spectral Parameters	MtxStd{GeneScan_SetG5_BT5}.par (parameters created before)

- Click into the column header to select the entire column, select **Edit** → **Fill Down** to apply the information of the selected samples and confirm with **OK**
- Link your reaction plate on the autosampler tray with the created plate ID and start run
- On completion of the run check in the **Spectral Calibration Result** dialog box if all capillaries have successfully passed calibration (label **A**). If individual capillaries are labelled **X**, refer to *ABI PRISM® Genetic Analyzer User's Manual*.
- Click on **OK** to confirm completion of the run

Matrix check

- Select **Tools → Display Spectral Calibration → Dye Set → G5** to review the spectral calibration profile for each capillary
- The quality value (**Q value**) must be greater than 0.95 and the condition number (**C value**) must be between 1 and 20. Both values must be within the previously determined range
- Check the matrix samples for a flat baseline. There should be five peaks with peak heights of about 1000-5000 RFU (Y-axis) in each matrix sample (optimal range: 2000-4000 RFU)
- If all capillaries have passed the calibration, the last calibration file for **Dye Set G5** must be activated manually under **Tools → Set Active Spectral Calibration**. Rename the calibration file under **Set Matrix Name** (e.g. BT5_Date of calibration)
- If calibration was not successful, try to re-inject the samples with higher injection voltage or injection time. The editing of the Spectral Run Module will be necessary. You can re-inject the same samples up to three times. Otherwise use more matrix standard for spectral calibration
- Check the new matrix with your current samples. There should be no pull-up peaks between the dye panels (B, G, Y, R, O) with the new matrix

4.2 Sample preparation

Component	Volume
Hi-Di™ Formamide	12.0 µl
DNA Size Standard 550 (BTO)	0.5 µl
Prepare 12 µl of the mix (formamide + DNA size standard) for all samples	
Add 1 µl PCR product (diluted if necessary) or allelic ladder	

- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place the samples on the autosampler tray

Since injections take place simultaneously on all capillaries, 4 or 16 samples must be pipetted on the plate of multi-capillary analyzers. If fewer samples are analysed, the empty positions must be filled with 12 µl Hi-Di™ Formamide.

To ensure a reliable allelic assignment on multi-capillary analyzers, several ladders should be run.

Room temperature may influence the performance of PCR products on multi-capillary instruments, so that shoulder peaks or split peaks occur especially at low temperatures. Pay attention to keeping ambient conditions as recommended by the instrument manufacturer. Optimal will be a stable room temperature > 22 °C.

Signal intensities

Options to increase the signal intensity:
- Reduce the volume of the DNA Size Standard 550 (BTO) to peak heights of about 500 relative fluorescent units (RFU)
- Purify the PCR products before starting the analysis

4.3 Setting up the Data Collection Software

Edit the default run module in **Dye Set G5** once for the first run.
- Select **Module Editor** to open the dialog box
- Select the appropriate **Run Module** as template from the **GeneScan** table
- Modify the **Injection Voltage** to 3 kV and the **Injection Time** to 10 s

Run Module 3kV_10s_450bp

Parameter	Set up
Run Temperature [°C]	*Default*
Cap Fill Volume	*Default*
Maximum Current [A]	*Default*
Current Tolerance [A]	*Default*
Run Current [A]	*Default*
Voltage Tolerance [kV]	*Default*
Pre Run Voltage [kV]	*Default*
Pre Run Time [s]	*Default*
Injection Voltage [kV]	3.0
Injection Time [s]*	10
Run Voltage [kV]	*Default*
Number of Steps	*Default*
Voltage Step Interval	*Default*
Data Delay Time [s]	*Default*
Run Time [min]**	25

* Deviating from the standard settings, the injection time may range between 1 and 20 s depending on the type of sample. If reference samples with very high signal intensities are recorded, a shorter injection time may be selected in order to avoid pull-up peaks. For samples with low DNA content an injection time of up to 20 s may be necessary.

** Depending on the analysis conditions, the Run Time for Mentype® **DIPscreen** was modified in order to be able to analyse fragments with lengths of up to **450 bp**.

- Click on **Save As**, enter the name of the new module (e.g. 3kV_10s_450bp) and confirm with **OK**
- Click on **Close** to exit the **Run Module Editor**

Starting the run

- Place the prepared 96-well plate on the autosampler tray
- Run the ABI PRISM® 3100 Data Collection software
- In **Plate View** click on **New** to open the **Plate Editor** dialog box
- Enter a name of the plate
- Select **GeneScan**
- Select **96–Well** as plate type and click on **Finish**

Plate Editor

Parameter	Set up
Sample Name	enter a name
Dyes	0
Colour Info	Ladder or sample
Project Name	e.g. 3100_Project1
Dye Set	G5
Run Module*	3kV_10s_450bp
Analysis Module 1	DefaultAnalysis.gsp

* parameter see above

- Complete the table in the **Plate Editor** and click on **OK**
- Click into the column header to select the entire column and select **Edit** → **Fill Down** to apply the information of the selected samples
- Link your reaction plate on the autosampler tray with the created plate ID and start the run
- On completion of the run, view data as **Color Data** in **Array View** of the 3100 Data Collection software or as **Analyzed Sample Files** under D:/AppliedBio/3100/DataExtractor/ExtractRuns

4.4 Analysis parameter / analysis method

The recommended analysis parameters are:

Analysis Range	Full Range
Data Processing	Baseline: Checked Multicomponent: Checked Smooth Options: Light
Peak Detection	Peak Amplitude Thresholds B:* Y:* G:* R:* O:* Min. Peak Half Width: 2 pts Polynominal Degree: 3 Peak Window Size: 15 pts
Size Call Range	Min: 60 Max: 550
Size Calling Method	Local Southern Method
Split Peak Correction	None

* The peak amplitude threshold (cutoff value) corresponds to the minimum peak height that will be detected by the GeneScan® or GeneMapper® ID software. Thresholds are usually 50-200 RFU and should be determined individually by the laboratory. Recommendation: The minimal peak height should be three times as high as the background noise of the baseline.

5. Electrophoresis using the ABI PRISM® 3130/3130xl Genetic Analyzer

For detailed instructions on instrument setup, spectral calibration, or application of the ABI PRISM® Data Collection software version 3.0 and the GeneMapper® ID/ID-X software, refer to the *ABI PRISM ® 3130/3130xl Genetic Analyzers Getting Started Guide*.

The system with 4 capillaries is named ABI 3130, and the system with 16 capillaries is named ABI 3130xl.

The virtual **filter set Any5Dye** shall be used for the combined application of the five fluorescent labels **6–FAM**, **BTG**, **BTY**, **BTR**, **and BTO** (the matrix standard will be called **BT5** hereinafter).

Material

Capillary	36 cm Capillary Array for 3130/3130xl
Polymer	POP4® Polymer for 3130
Buffer	10x Genetic Analyzer Buffer with EDTA

5.1 Spectral calibration / matrix generation

Prior to conducting DNA fragment size analysis, it is necessary to perform a spectral calibration with the five fluorescent labels **6–FAM**, **BTG**, **BTY**, **BTR**, and **BTO** for each analyzer. The calibration procedure creates a matrix which is used to correct the overlapping of fluorescence emission spectra of the dyes.

Spectral calibration comprises the following steps:
- Preparation the spectral calibration standards
- Loading the standards to the 96-well reaction plate (one sample per capillary)
- Creating the instrument protocol for spectral calibration (Protocol Manager)
- Defining the plate composition in the plate editor (Plate Manager)
- Performing a spectral calibration run and checking the matrix

Setting up the spectral calibration standards

Example for 4 capillaries/ABI 3130

Component	Volume
Hi-Di™ Formamide	60.0 µl
Matrix standard **BT5**	5.0 µl

- Load 12 µl of the mix to a 96-well reaction plate, e.g. position **A1–D1**
- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place samples in the autosampler tray

Example for 16 capillaries/ABI 3130xl

Component	Volume
Hi-Di™ Formamide	204.0 µl
Matrix standard **BT5**	17.0 µl

- Load 12 µl of the mix to a 96-well reaction plate, e.g. position **A1–H1** and **A2–H2**
- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place samples in the autosampler tray

Performing a spectral calibration run

- Place the 96-well plate on the autosampler tray
- In the **Protocol Manager** of the Data Collection software click on **New** in **Instrument Protocol** to open the **Protocol Editor** dialog box

Instrument Protocol for spectral calibration

Protocol Editor	Set up
Name	*User* (e.g. Spectral36_POP4_BT5)
Type	SPECTRAL
Dye Set	**Any5Dye**
Polymer*	*User* (e.g. POP4)
Array Length*	*User* (e.g. 36cm)
Chemistry	Matrix Standard
Run Module*	*Default* (e.g. Spect36_POP4_1)

* Depends on the type of polymer and length of capillary used

- Click on **OK** to leave the **Protocol Editor** dialog box
- In the **Plate Manager** of the Data Collection software, click on **New** to open the **New Plate Dialog** box

Plate Editor for spectral calibration (I)

New Plate Dialog	Set up
Name	e.g. Spectral_BT5_date
Application	Spectral Calibration
Plate Type	96-Well
Owner Name / Operator Name	…

- Click on **OK**. A new table in the **Plate Editor** will open automatically

Plate Editor for spectral calibration (II)

Parameter	Set up
Sample Name	Enter name for the matrix samples
Priority	e.g. 100
Instrument Protocol 1	Spectral36_POP4_BT5 (setting described before)

- Click into the column header to select the entire column, select **Edit** → **Fill Down** to apply the information to all selected samples, and click on **OK**
- In the **Run Scheduler** click on **Find All**, select **Link** to link the reaction plate on the autosampler up with the newly created plate record (position A or B) and start the run

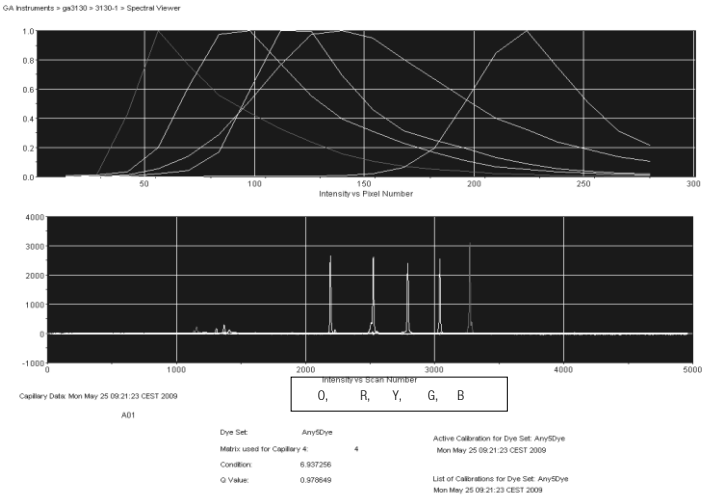


Fig. 4 Electropherogram of spectral calibration with matrix standard BT5 on an ABI 3130

Matrix check

- The quality value (**Q value**) of each capillary must be greater than 0.95 and the condition number range (**C value**) must be between 1 and 20
- Check the matrix samples for a flat baseline. As shown in the figure above, there should be five peaks with peak heights of about 1000-5000 RFU (Y-axis) in each matrix sample (optimal range: 2000-4000 RFU)
- If all capillaries have passed the test, the last calibration file for the Dye Set **Any5Dye** is activated automatically in the **Spectral Viewer**. **Rename** the calibration file (e.g. BT5_Date of calibration) using the respective button
- If calibration was not successful, try to re-inject the samples with higher injection voltage or injection time. Editing of the Spectral Run Module will be necessary. You can re-inject the same samples up to three times. Otherwise use more matrix standard for spectral calibration
- Check the new matrix with your current samples. There should be no pull-up peaks between the dye panels (B, G, Y, R, O) with the new matrix

Sample preparation

Component	Volume
Hi-Di™ Formamide	12.0 µl
DNA Size Standard 550 (BTO)	0.5 µl
Prepare 12 µl of the mix (formamide + DNA size standard) for all samples	
Add 1 µl PCR product (diluted if necessary) or allelic ladder	

- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place the samples on the tray

Since injections take place simultaneously on all capillaries, 4 or 16 samples must be pipetted on the plate of multi-capillary analyzers. If fewer samples are analysed, the empty positions must be filled with 12 µl Hi-Di™ Formamide.

To ensure a reliable allelic assignment on multi-capillary analysers, several ladders should be run.

Room temperature may influence the performance of PCR products on multi-capillary instruments, so that shoulder peaks or split peaks occur especially at low temperatures. Pay attention to keeping ambient conditions as recommended by the instrument manufacturer. Optimal will be a stable room temperature > 22 °C.

Signal intensities

Options to increase the signal intensity:

- Reduce the volume of the DNA Size Standard 550 (BTO) to peak heights of about 500 relative fluorescent units (RFU)
- Purify the PCR products before starting the analysis

5.2 Setting up the Data Collection Software

Edit the run module as follows for the first run:

- In the **Module Manager** of the Data Collection Software click on **New** to open the **Run Module Editor** dialog box

Run Module 3kV_10s_450bp

Parameter	Set up
Oven Temperature [°C]	*Default*
Poly Fill Volume	*Default*
Current Stability [µA]	*Default*
PreRun Voltage [kV]	*Default*
PreRun Time [s]	*Default*
Injection Voltage [kV]	3.0
Injection Time [s]*	10
Voltage Number of Steps	*Default*
Voltage Step Interval	*Default*
Data Delay Time [s]	*Default*
Run Voltage [kV]	*Default*
Run Time [s]**	1500

* Deviating from the standard settings, the injection time may range between 1 and 20 s depending on the type of sample. If references samples with very high signal intensities are recorded, a shorter injection time may be selected in orer to avoid pull-up peaks. For samples with low DNA content an injection time of up to 20 s my be necessary.

** Depending on the analysis conditions the run time for Mentype® **DIPscreen** was modified in order to be able to analyse fragments with lengths of up to **450 bp**.

- Click on **Save As**, enter the name of the new module (e.g. 3kV_10s_450bp) and confirm with **OK**
- Click on **Close** to exit the **Run Module Editor**

Starting the run

- Place the prepared 96-well plate on the autosampler tray
- In the **Protocol Manager** of the Data Collection software, click on **New** in the **Instrument Protocol** window to open the **Protocol Editor** dialog box

Instrument Protocol

Protocol Editor	Set up
Name	enter a name
Type	REGULAR
Run Module*	3kV_10s_450bp
Dye Set	**Any5Dye**

* parameter see above

- Click on **OK** to exit the **Protocol Editor**

Prior to each run, it is necessary to create a plate definition as follows:
- In the **Plate Manager** of the Data Collection software click on **New** to open the **New Plate Dialog** box

Plate Editor (I)

New Plate Dialog	Set up
Name	e.g. Plate_BT5_Date
Application	Select GeneMapper Application
Plate Type	96-Well
Owner Name / Operator Name	…

- Click on **OK**. A new table in the **Plate Editor** will open automatically

Plate Editor (II)

Parameter	Set up
Sample Name	Enter name for the samples
Priority	e.g. 100 (Default)
Sample Type	Sample or allelic ladder
Size Standard	e.g. SST-BTO_60-450bp
Panel	e.g. DIPscreen_Panels_v0
Analysis Method	e.g. Analysis_DIPscreen_3130_200rfu
Snp Set	-
User-defined 1-3	-
Results Group 1	(select results group)
Instrument Protocol 1	Run36_POP4_BT5_25min (setting described before)

- Click into the column header to select the entire column, select **Edit → Fill Down** to apply the information to all selected samples and click on **OK**
- In the **Run Scheduler**, click on **Find All**, select **Link** to link the reaction plate on the autosampler up with the newly created plate record (position A or B) and start the run
- During the run, view **Error Status** in the **Event Log** or examine the quality of the raw data for each capillary in the **Capillaries Viewer** or the **Cap/Array Viewer**
- View data as overview in **Run History** or **Cap/Array Viewer** of the Data Collection software. Run data are saved in the **Run Folder** of the previously chosen **Result Group**

5.3 Analysis parameter / analysis method

The recommended analysis parameters are:

Peak Detection Algorithm	Advanced
Ranges	Analysis: Full Range Sizing: All Sizes
Smoothing and Baselining	Smoothing: Light Baseline Window: 51 pts
Size Calling Method	Local Southern Method
Peak Detection	Peak Amplitude Thresholds B:* Y:* G:* R:* O:* Min. Peak Half Width: 2 pts Polynominal Degree: 3 Peak Window Size: 15 pts Slope Thresholds: 0.0

Recommend settings in the worksheet **Allele** are:

Amelogenin Cutoff**	0.1

Recommend settings in the worksheet **Peak Quality** are:

Heterozygote balance	Min peak height ratio: 0.1
Allele number	Max expected alleles: 2

* The peak amplitude threshold (Cutoff value) corresponds to the minimum peak height that will be detected from the GeneMapper® ID/ID-X software. The thresholds are usually 50-200 RFU and should be determined individually by the laboratory. Recommendation: The minimal peak height should be three times higher then the background noise of the baseline.

** All DIPs will be examined by GeneMapper® ID/ID-X software like Amelogenin.

6. Electrophoresis using the ABI PRISM® 3500/3500xL Genetic Analyzer

For detailed instructions on instrument setup, spectral calibration, or application of the Applied Biosystems 3500 Series Data Collection Software version 1.0 and the GeneMapper® ID-X software version 1.2, refer to the *Applied Biosystems 3500/3500xL Genetic* Analyzers User Guide.

The system with 8 capillaries is named AB 3500 and the system with 24 capillaries is named AB 3500xL.

The virtual **filter set Any5Dye** shall be used for the combined application of five fluorescent labels **6–FAM**, **BTG**, **BTY**, **BTR**, and **BTO** (the matrix standard will be called **BT5** hereinafter).

Material

Capillary	36 cm Capillary Array for 3500/3500xL
Polymer	POP-4® Polymer for 3500/3500xL
Buffer	10x Genetic Analyzer Buffer with EDTA for 3500/3500xL

6.1 Spectral calibration / matrix generation

Prior to conducting DNA fragment size analysis, it is necessary to perform a spectral calibration with the fluorescent labels **6–FAM**, **BTG**, **BTY**, **BTR**, and **BTO** for each analyzer. The calibration procedure creates a matrix that is used to correct the overlap of fluorescence emission spectra of the dyes.

Spectral calibration comprises the following steps:
- Preparation of spectral calibration standards
- Loading the standards to the multi-well reaction plate (one sample per capillary)
- Preparation of instrument and creating a Dye Set BT5
- Performing a spectral calibration run and checking the matrix

Setting up the spectral calibration standards

Example for 8 capillaries/ABI 3500

Component	Volume
Hi-Di™ Formamide	108.0 µl
Matrix standard **BT5**	9.0 µl

- Load 12 µl of the mix to a 96-well reaction plate, e.g. position **A1–H1**
- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place samples in the autosaple tray

Example for 24 capillaries/ABI 3500xL

Component	Volume
Hi-Di™ Formamide	300.0 µl
Matrix standard **BT5**	25.0 µl

- Load 12 µl of the mix to a 96-well reaction plate, e.g. position **A1–H1**, **A2–H2 and A3–H3***
- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place samples in the autosample tray

* When using a 384-well plate, load 10 µl of the mixtures to columns 1, 3, and 5 in rows A, C, E, G, I, K, M, and O.

Performing a spectral calibration run

- Place the multi-well plate on the autosampler tray
- Now prepare the instrument and specific spectral calibration run settings

Preparation of the instrument

Before starting the spectral calibration process ensure that the spatial calibration has been performed. This process is necessary if a new capillary array was installed before and is described in detail in the *Applied Biosystems 3500/3500xL Genetic Analyzers User Guide*.

Preparation of dye set BT5

Prior to the spectral calibration, a dye set for the Matrix Standard BT5 needs to be setup.

1. To create a new dye set, go to **Library** and select **Analyze**, followed by **Dye Sets** and click **Create**.
2. Enter a **Dye Set Name**, e.g. BT5.
3. Select **Matrix Standard** as a chemistry and **AnyDye Template** as a Dye Set Template.
4. Disable **Purple** in the field **Arrange Dyes**. Ensure that all other colors are enabled.
5. Under **Calibration Peak Order** the colors need to be arranged as Follows: **5 – blue**, **4 – green**, **3 – yellow**, **2 – red**, and **1 – orange**.
6. Do not alter the **Parameter** settings.
7. Click **Save** to confirm the changes.

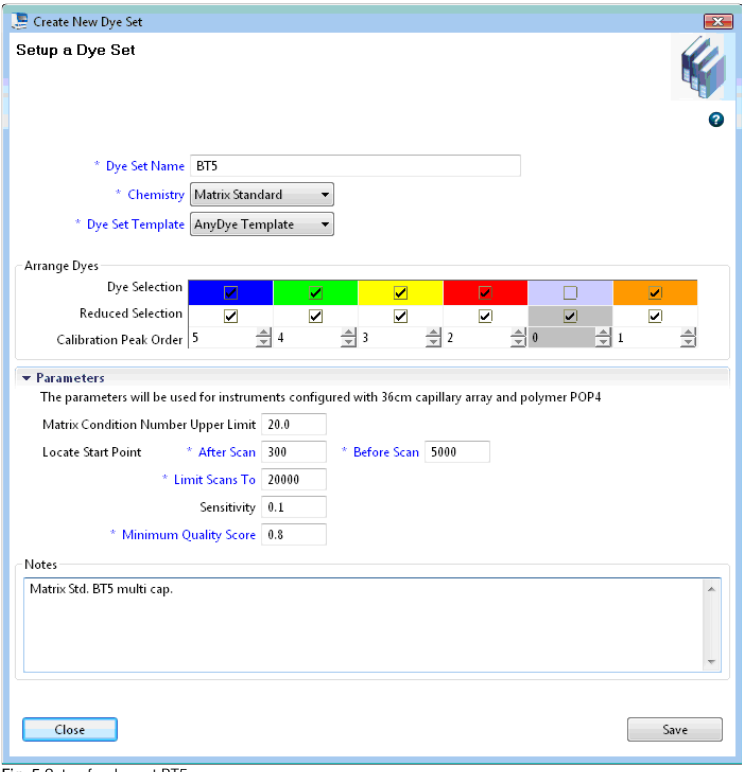


Fig. 5 Setup for dye set BT5

- In the **Protocol Manager** of the Data Collection software click on **New** in **Instrument Protocol** to open the **Protocol Editor** dialog box

Performing a spectral calibration run

Once the multiwell plate containing the spectral calibration mixture is placed in the autosampler tray the spectral calibration process can be started.

1. To access the Spectral Calibration screen, select **Maintenance** on the Dashboard of the 3500 Series Data Collection software.
2. The number of wells in the spectral calibration plate and their location in the instrument must be specified.
3. Select **Matrix Standard** as a chemistry standard and **BT5** for dye set.
4. (Optional) Enable **Allow Borrowing**.
5. Click **Start Run**.

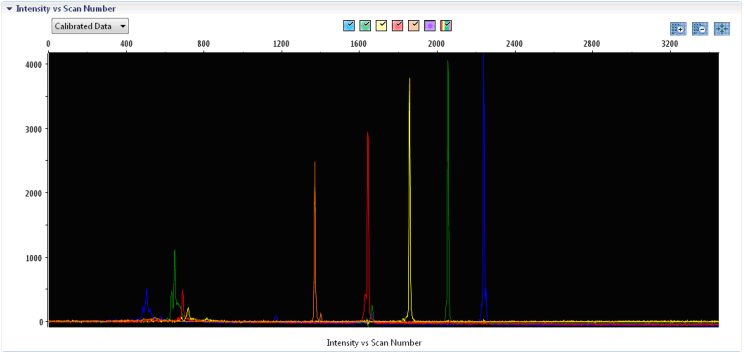


Fig. 6 Electropherogram of spectral calibration with matrix standard BT5 on an ABI 3500

Matrix check

- The quality value (**Q value**) of each capillary must be greater than 0.8 and the condition number range (**C value**) must be between 1 and 20
- Check the matrix samples for a flat baseline. As shown in the figure above, there should be five peaks with peak heights of about 1000-5000 RFU (Y-axis) in each matrix sample (optimal range: 2000-4000 RFU)
- A successful calibration will be displayed in green in **Overall** and for each capillary
- If all capillaries have passed the test, **Accept Results**
- If calibration failed, **Reject Results** and refer to **spectral calibration troubleshooting** of Applied Biosystems 3500/3500xL Genetic Analyzer User Guides

6.2 Sample preparation

Component	Volume
Hi-Di™ Formamide	12.0 µl
DNA Size Standard 550 (BTO)	0.5 µl
prepare 12 µl of the mix (formamide + DNA size standard) for all samples add 1 µl PCR product (diluted if necessary) or allelic ladder	
- Denaturation for 3 min at 95 °C - Cool down to 4 °C and place the samples on the autosample tray	

Since injections take place simultaneously on all capillaries, 8 or 24 samples must be pipetted on the plate of multi-capillary analysers. If fewer samples are analysed empty positions need to be filled with 12 µl Hi-Di™ Formamide.

To ensure a reliable allelic assignment on multi-capillary analysers, several ladders should be run.

Room temperature may influence the performance of PCR products on multi-capillary instruments, so that shoulder peaks or split peaks occur especially at low temperatures. Pay attention to keeping ambient conditions as recommended by the instrument manufacturer. Optimal will be a stable room temperature > 22 °C.

Signal intensities

Options to increase the signal intensity:

- Reduce the volume of the DNA Size Standard 550 (BTO) to peak heights of about 500 relative fluorescent units (RFU)
- Purify the PCR products before starting the analysis

6.3 Setting up a run

For the first run using the Mentype® **DIPscreen** Kit you will need to setup a number of protocols within the 3500 Series Data Collection Software.

Create Instrument protocol

- Go to **Library** and select **Analyze** / **Instrument protocol** and click **Create**
- Change the parameters according the table below

Instrument protocol for Mentype® DIPscreen®

Parameter	Set up
Application Type	HID / Microsatellite
Capillary Length	*Default*
Polymer	*Default*
Dye Set	BT5
Run Module	*Default*
Protocol Name	e.g. Mentype DIPscreen
Oven Temperature [°C]	*Default*
Run Voltage [kV]	*Default*
Injection Voltage [kV]	3.0
Run Time [s]**	1500
PreRun Time [s]	*Default*
Injection Time [s]*	10
Data Delay Time [s]	*Default*
Advanced Options	*Default*

* Deviating from the standard settings, the injection time may range between 1 and 20 s depending on the type of sample. If reference samples with very high signal intensities are recorded, a shorter injection time may be selected in order to avoid pull-up peaks. For samples with low DNA content an injection time of up to 20 s may be necessary.

** Depending on the analysis conditions, the run time for Mentype® **DIPscreen** was modified in order to analyse fragments with lengths of up to **450 bp**.

- Click on **Save** to confirm the settings

Create Size Standard

- Go to **Library** and select **Analyze** / **Size Standards** and click **Create**
- Change the parameters according the table below

Parameter	Set up
Size Standard	BTO_550
Dye Color	Orange

The DNA Size Standard 550 (BTO) should be used with the following lengths of fragments: **60**, **80**, **90**, **100**, **120**, **140**, **160**, **180**, **200**, **220**, **240**, **250**, **260**, **280**, **300**, **320**, **340**, **360**, **380**, **400**, **425**, **450**, **475**, **500**, **525**, and **550** bp.

- Click on **Save** to confirm the settings

Create QC (Size Calling) Protocol

- Go to **Library** and select **Analyze** / **QC (Size Calling)** and click **Create**
- Change the parameters according the table below

Parameter	Set up
Protocol Name	enter a name
Size Standard	BTO_550 (from above)
Sizecaller	Size Caller v.1.1.0

- Go to **Analysis Settings** / **Peak Amplitude Threshold** and **disable purple**. All other colours should be enabled.
- Keep all other settings as Default
- Click on **Save** to confirm the settings

Create an Assay

- Go to **Library** and select **Manage** / **Assays** and click **Create**
- Change the parameters according the table below

Parameter	Set up
Assay Name	e.g. Mentype DIPscreen
Color	Default
Application Type	HID
Instrument Protocol	e.g. Mentype DIPscreen
QC Protocols	e.g. BTO_550

- Click on **Save** to confirm the settings

Starting the run

- Place the prepared multi-well plate on the autosampler tray
- In the **Dashboard** of the Data Collection Software, click **Create New Plate**
- Go **to Define Plate Properties** and select **Plate Details**
- Change the parameters according the table below

Plate Details

Property	Set up
Name	enter a name
Number of Wells	96 or 384
Plate Type*	HID
Capillary Lenght	**36cm**
Polymer	**POP4**

- Click **Assign Plate Contents** to confirm the settings
- Define well position of each sample or ladder for data collection and processing by entering sample names
- Assign an **Assay** (required) a **File Name Conventions** and a **Result Group** to all named wells in the plate
- Click **Link the plate for Run** and enter Run Name
- Click **Start Run**

7. Analysis

For general instructions on automatic sample analysis, refer to the *GeneScan®* or *GeneMapper® ID or GeneMapper®ID-X Software User's Manual.*

Note: Within the Mentype® DIPscreen the red panel should be faded out.

Finding the exact lengths of the amplified products depends on the device type, the conditions of electrophoresis, as well as the DNA size standard used. The DNA Size Standard 550 (BTO) shall thus be used with the following lengths of fragments: **60**, **80**, **90**, **100**, **120**, **140**, **160**, **180**, **200**, **220**, **240**, **250**, **260**, **280**, **300**, **320**, **340**, **360**, **380**, **400**, **425**, **450**, **475**, **500**, **525**, and **550** bp.

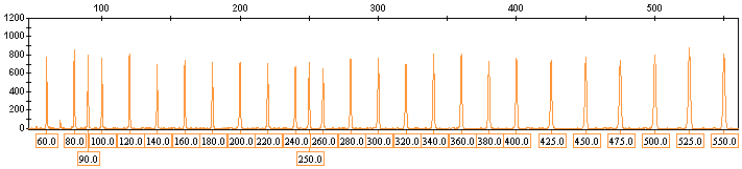


Fig. 7 Electropherogram of the DNA Size Standard 550 (BTO), fragments with lengths in bp

Note: The provided template files for the DNA size standard SST-BTO_60-450bp can be applied for the evaluation and analysis of the Mentype® **DIPscreen** using the GeneMapper® ID or ID-X Software.

7.1 Biotype® template files

Allele allocation should be carried out with suitable analysis software, e.g. the GeneMapper® ID/ID-X software in combination with the Mentype® DIPscreen template files from Biotype or the Chimeris™**Monitor** Software of Biotype Diagnostic GmbH. Template files are available from our homepage or as CD-ROM on request.

Recommended Biotype® templates for GeneMapper® ID/ID-X Software are:

Panels	DIPscreen_Panels_v0/v0X	or higher versions
BinSets	DIPscreen_Bins_v0/v0X	or higher versions
Size Standard	SST-BTO_60-450bp	
Analysis Method	Analysis_DIPscreen_310_200rfu	
	Analysis_DIPscreen_310_1000rfu	
	Analysis_DIPscreen_3130_200rfu	
	Analysis_DIPscreen_3130_1000rfu	
Plot Settings	PlotsBT5_4dyes	
Table Settings	Table for 2 alleles	

Panels and BinSets always have to be used whereas the other template files are optional.
The prepared Biotype® templates for GeneMapper® ID/ID-X Software were generated for POP4® runs. In case of using other polymer types changes may necessary on Panels and Bins or within the Analysis Method before analyzing the data.
For detailed instruction please refer to the instruction Biotype® Template Files für GeneMapper® which can be downloaded from our homepage (www.biotype.de).

Important Note: Import and allele calling with provided template files is only guarantied using GeneMapper® ID/ID-X software. If GeneMapper® software is applied you may experience import problems using some template files. You may have to adjust Panels and Bins with one ore more runs of the allelic ladder on your specific instrument setup. Contact us for support (support@biotype,de).

General procedure for the analysis

1. Check the DNA size standard
2. Check the allelic ladder
3. Check the positive control
4. Check the negative control
5. Analyse and interpret the sample data

7.2 Controls

The Control DNA XY13 of the test kit and other commercially available DNA from standard cell lines represent the following alleles:

Table 2. Allele determinations of Mentype ® DIPscreen

Locus	Control–DNA XY13	ATCC K–562	CCR 9947A	CCR 9948	CCR 3657
AM	XY	XX	XX	XY	XY
HLD106	+/+	-/-	+/+	+/+	+/+
HLD70	-/+	-/+	+/+	-/+	-/-
HLD84	-/+	+/+	-/-	-/+	-/-
HLD103	+/+	-/-	-/+	+/+	-/+
HLD104	-/+	-/-	-/+	+/+	-/-
HLD116	-/+	+/+	-/-	-/+	-/-
HLD112	-/+	+/+	-/+	-/+	-/+
HLD307	+/+	+/+	-/+	+/+	+/+
HLD310	+/+	-/+	-/+	-/-	-/+
HLD110	-/+	-/+	-/+	-/+	-/+
HLD133	-/+	-/-	+/+	+/+	-/+
HLD79	+/+	+/+	+/+	-/+	+/+
HLD105	-/+	-/-	-/+	-/+	-/+
HLD140	+/+	+/+	-/-	-/+	+/+
HLD163	+/+	-/+	-/+	+/+	-/+
HLD91	-/+	-/+	-/-	-/-	-/+
HLD23	-/+	+/+	-/-	-/+	-/+
HLD88	+/+	-/-	-/-	-/+	+/+
HLD101	-/+	-/+	-/+	-/+	-/+
HLD67	-/+	-/+	+/+	+/+	+/+
HLD301	-/+	-/+	-/+	-/+	-/-
HLD53	+/+	-/-	-/+	+/+	-/-
HLD97	-/-	-/-	-/+	-/+	+/+
HLD152	-/-	+/+	+/+	-/+	+/+
HLD128	-/+	-/+	-/+	-/-	-/+
HLD134	-/+	-/-	+/+	+/+	-/-
HLD305	-/+	-/+	-/+	+/+	-/+
HLD48	-/+	+/+	+/+	-/+	+/+
HLD114	+/+	-/-	-/-	+/+	-/+
HLD304	+/+	-/-	-/+	-/+	-/-
HLD131	+/+	-/+	-/-	-/+	+/+
HLD38	+/+	-/+	-/+	+/+	+/+
HLD82	+/+	+/+	+/+	-/+	+/+

The reference DNA K-562 is available from ATCC (http://atcc.org/Produtcs/PurifiedDNA.cfm#celllines), DNA 9947A, 9948 and 3657 are available from Coriell Cell Repositories (CCR; http://locus.umdnj.edu/nigms/) .

7.3 Lengths of fragments and alleles

Table 3 show the fragment lengths of individual alleles that refer to the DNA Size Standard 550 (BTO). All analyses have been performed on an ABI PRISM® 3130 Genetic Analyzer with POP4® polymer. Different analysis instruments, DNA size standards or polymers may result in different fragment lengths. In addition, a visual alignment with the allelic ladder is recommended.

Scaling

Horizontal: 70-430bp (see Fig. 8 and 9)
Vertical: Depending on signal intensity

Figure 8

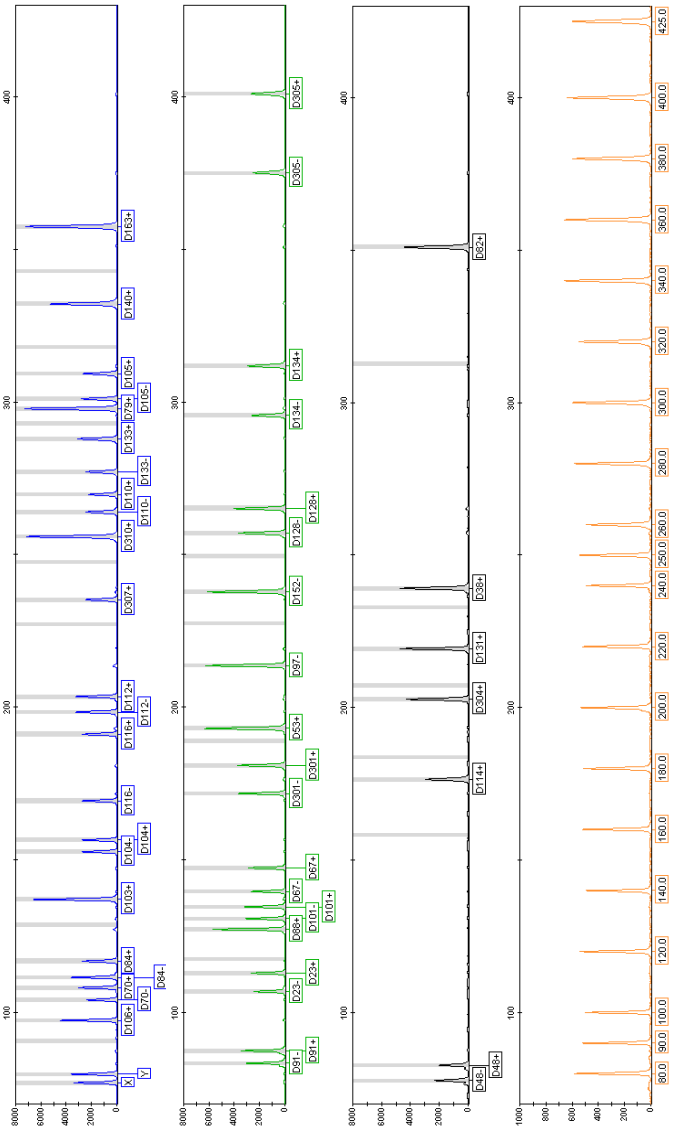


Fig. 8 Electropherogram of the Mentype® **DIPscreen** using 1ng Control DNA XY13. Analysis was performed on an ABI PRISM® 3130 Genetic Analyzer with the DNA Size Standard 550 (BTO). Allele assignment was performed using the GeneMapper® ID Software and the Mentype® **DIPscreen** template file.

Figure 9

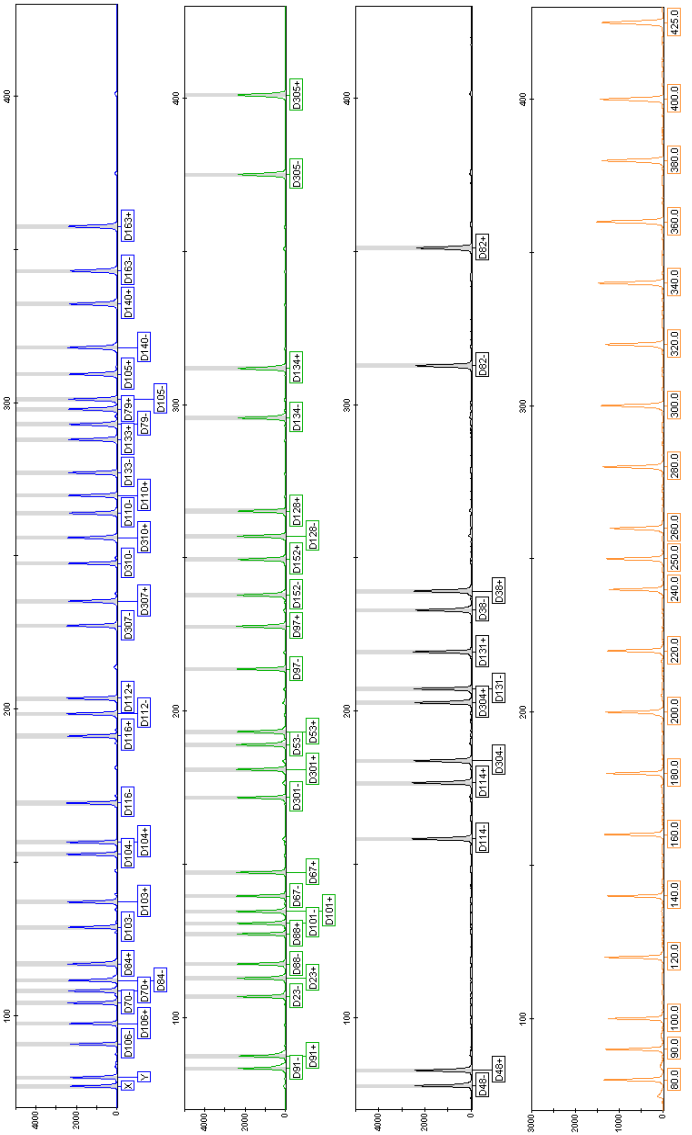


Fig. 9 Electropherogram of the allelic ladder Mentype® **DIPscreen**. Analysis was performed on an ABI PRISM® 3130 Genetic Analyzer with the DNA Size Standard 550 (BTO). Allele assignment was performed using the GeneMapper® ID Software and the Mentype® **DIPscreen** template file.

Table 3. Fragment lengths of the Mentype® DIPscreen allelic ladder analysed on an ABI PRISM® 3130 Genetic Analyzer with POP4® (FAM, BTG, BTY panel)

Marker/FAM	–DIP [bp]*	+DIP [bp]*	Marker/BTG	–DIP [bp]*	+DIP [bp]*
AM	77 (X)	80 (Y)	HLD91	84	88
HLD106	91	98	HLD23	107	113
HLD70	104	108	HLD88	118	128
HLD84	112	117	HLD101	131	135
HLD103	129	138	HLD67	140	148
HLD104	153	1157	HLD301	172	182
HLD116	170	192	HLD53	190	194
HLD112	199	204	HLD97	214	228
HLD307	228	236	HLD152	239	250
HLD310	248	257	HLD128	258	266
HLD110	264	270	HLD134	296	312
HLD133	278	288	HLD305	375	401
HLD79	294	299			
HLD105	302	310	Marker/BTY	–DIP [bp]*	+DIP [bp]*
HLD140	318	333	HLD48	78	83
HLD163	344	358	HLD114	159	177
			HLD304	184	203
			HLD131	208	220
			HLD38	234	240
			HLD82	314	352

* rounded to integer

8. Interpretation of results

As mentioned above, post PCR analysis and automatic allele allocation with suitable analysis software ensure a precise and reliable discrimination of alleles.

The automated identification of informative DIP-loci directly from raw data of fragment analysis runs and the selection of suitable DIPquant assays for monitoring could be performed by using Chimeris™**Monitor** Software from Biotype Diagnostic GmbH.

Pull–up peaks

Pull-up peaks may occur if peak heights are outside the linear detection range (>3000 RFU), or if an incorrect matrix was applied. They appear at positions of specific peaks in other colour channels, typically with lower signal intensities. Peak heights should not exceed 3000 RFU in order to prevent pull-up peaks.

Template–independent addition of nucleotides

Because of its terminal transferase activity, the Multi Taq DNA Polymerase tends to add an adenosine radical at the 3'-end of the amplified DNA fragments. The artefact peak is one base shorter than expected (-1 bp peaks). All Biotype® primers are designed to minimise these artefacts. Artefact formation is further reduced by the final extension step of the PCR protocol at 68 °C for 60 min. Peak height of the artefact correlates with the amount of DNA. Laboratories should define their individual limits for analysis of the peaks.

Artefacts

Room temperature may influence the performance of PCR products on multi-capillary instruments, shoulder peaks or split peaks occur. Furthermore, automated assignment could be influenced in some cases. If these effects occur we recommend injecting the sample again at higher room temperature and maybe using more than one allelic ladder sample per run.

Influence of polymers

Mentype® **DIPscreen** was validated and certified for the analysis on POP4® polymer. The use of other polymers (e.g. POP7™ or POP6™) might influence the run behaviour of specific PCR products. Furthermore background noise might increase through different behaviour of free fluorescent dyes.

9. References

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10. Explanation of Symbols

Manufacturer

Date of manufacture

Batch code

Contains sufficient reagents for <N> tests

Consult instructions (handbook) for use

Use by

Temperature limitations

Catalogue number

In–Vitro–Diagnostics

Notes

Notes